

Improving Libido and Sperm Quality of Bali Bulls by Supplementation of *Moringa oleifera* Leaves

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ABSTRACT

Moringa oleifera (Moringa) leaves contain high levels of nutrient that can be utilized as a feed supplement to increase libido and semen quality. This study was aimed to evaluate the effect of *Moringa* leaves supplementation on libido and sperm quality of Bali bulls. This study used two-sample t-test on four Bali bulls were kept under individual pens for two periods of eight weeks. During the first period (control), the experimental Bali bulls were fed concentrates (1% of body weight) and rice straw *ad libitum*. During the second period, the experimental Bali bulls were fed similar to the first period with an additional of *Moringa* leaves 15% of the weight of the concentrate (treatments). Libido and sperm quality were measured twice a week. Plasma samples were taken three times a day (06:00, 14:00, and 22:00h) on the last day of each period. Plasma testosterone concentrations were analyzed by using ELISA techniques. Variables measured were compared using paired student's t-test. Supplementation of *Moringa* leaves increased testosterone levels ($P<0.05$) (4.57 vs 4.79, 0.45 vs 4.78, and 2.35 vs 5.63 ng/mL, respectively during 06.00; 14.00; and 22.00h), increased libido ($P<0.05$) (7.20 \pm 1.49 vs 3.49 \pm 0.40 min), and increased both the total motility ($P<0.05$) (63.99 \pm 3.37% vs 84.96 \pm 3.09%) and the progressive motility ($P<0.05$) (52.77 \pm 1.76% vs 67.03 \pm 3.74%) of sperm. Supplementation of *Moringa* leaves also increased ($P<0.05$) the velocity and the amplitude and decreased ($P<0.05$) the linearity of sperm. It can be concluded that the supplementation of *Moringa* leaves could increase plasma testosterone concentrations, libido, and sperm motility of Bali bulls.

Keywords: libido, semen quality, Bali bulls, *Moringa oleifera*, rice straw

ABSTRAK

Daun kelor mengandung nutrisi yang tinggi sehingga dapat dimanfaatkan sebagai bahan pakan tambahan untuk meningkatkan libido dan kualitas semen. Penelitian ini bertujuan untuk mengevaluasi pengaruh suplementasi daun kelor terhadap libido dan kualitas sperma pejantan sapi Bali. Penelitian ini menggunakan uji perbandingan dua nilai tengah terhadap 4 ekor pejantan sapi Bali yang dipelihara secara intensif pada kandang individual selama 2 periode, masing-masing 8 minggu. Pada periode pertama (kontrol), sapi pejantan diberi pakan konsentrat 1% bobot badan dan jerami padi secara *ad libitum*. Pada periode kedua, sapi pejantan tersebut diberi pakan yang sama dengan pada periode pertama dengan tambahan daun kelor 15% dari bobot konsentrat (perlakuan). Libido dan kualitas sperma diukur 2 kali seminggu. Sampel plasma diambil 3 kali sehari (pukul 06.00, 14.00, dan 22.00) pada hari terakhir setiap periode. Konsentrasi testosteron dalam plasma dianalisis dengan menggunakan teknik ELISA. Peubah yang diukur dibandingkan dengan menggunakan uji *t* berpasangan (paired student's t-test). Suplementasi daun kelor meningkatkan kadar hormon testosteron dalam plasma ($P<0,05$) (4,57 vs 4,79; 0,45 vs 4,78; dan 2,35 vs 5,63 ng/mL), masing-masing pada pukul 06.00, 14.00, dan 22.00), meningkatkan libido ($P<0.05$) (7,20 \pm 1,49 vs 3,49 \pm 0,40 menit), dan meningkatkan motilitas total ($P<0.05$) (63,99 \pm 3,37% vs 84,96 \pm 3,09%) dan motilitas progresif ($P<0.05$) (52,77 \pm 1,76% vs 67,03 \pm 3,74%) sperma pejantan sapi Bali. Suplementasi daun kelor juga meningkatkan ($P<0,05$) kecepatan dan amplitudo, serta menurunkan ($P<0,05$) linearitas sperma. Dapat disimpulkan bahwa suplementasi daun kelor dapat meningkatkan kadar hormon testosteron dalam plasma, libido, dan motilitas sperma pejantan sapi Bali.

Kata kunci: libido, kualitas air mani, pejantan sapi Bali, *Moringa oleifera*, jerami padi

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INTRODUCTION

Low libido and poor semen quality are factors affecting the reproductive performance of bulls. The bulls to be used for natural mating and as sources of semen for artificial insemination should have good libidos and semen qualities, because these factors will affect the reproductive efficiency of cows. Subfertile bulls like low libido and poor semen quality will delay conception, prolong the calving season, reduce calf weaning weights, and increase the numbers of cows culled, thereby resulting in economic losses and threatening the sustainability of a livestock operation (Kastelic, 2013).

The sexual behavior (libido and mounting capacity) of bulls depends on social interaction, which is affected by genetic, environmental, nutritional, and hormonal factors, as well as by sensorial acuity, age, and experience (Menegassi *et al.*, 2011). The semen quality of a bull is influenced by the nutritional factor (Martin *et al.*, 2010), age, season (Bhakat *et al.*, 2011), and breed (Lemma & Shemsu, 2015). The nutrition status of a bull controls sperm production, the secretion of gonadotropin, and sexual development. The capacity of testes of an adult male animal to produce sperm and testosterone is influenced by the ability of the seminiferous tubules and the Leydig cells or interstitial cells under the stimulation of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) (Martin *et al.*, 2010).

The process of spermatogenesis to produce semen of a good quality requires amino acids methionine, cysteine (Young *et al.*, 2008), and arginine (Wu *et al.*, 2009), a fatty acid α -linoleic, vitamin A, C, and E as well as Zn and Se (Cheah & Yang, 2011). Zn plays a fundamental role in the production of many sex hormones including testosterone and Gonadotropin Releasing Hormone (GnRH). Zn stimulates Leydig cells of the testis to produce testosterone. It is localized in Golgi complex or secretory vesicles of interstitiocytes, folliculocytes, and lactocytes of the pituitary gland. This element plays an important role in the production and secretion of FSH, LH, and prolactin that eventually regulate testosterone production (Roy *et al.*, 2013). The bio-constituents contained in herbs that are responsible for enhancing sexual activity and spermatogenesis include saponin, alkaloid, flavonoid, ferulic acid, and chlorogenic acid (Chauhan *et al.*, 2014). One of the plants that contain all of these compounds is *Moringa oleifera* leaves.

M. oleifera is a tree plant, found in the tropical countries, that is potential to be used for animal feed. *Moringa* leaves is a good alternative for fodder crops, especially in the dry season when no fodder is available (Nouman *et al.*, 2013). The biomass production is 4.2-8.2 tons DM/ha and is a good alternative for substituting commercial ration for livestock (Nouman *et al.*, 2014) and a good feed additive (Fitri *et al.*, 2015). The leaves of this plant are rich in nutritive values. The values of amino acids, fatty acids, minerals, and vitamin profiles reflect a desirable nutritional balance. The levels of crude protein content is 30.29%, fat is 6.50%, ash is 7.64%, calcium is 3.65%, phosphorus is 0.3%, neutral detergent fibre is 11.4%, acid detergent fibre is 8.49%, acid detergent lignin is 1.80%, acid detergent cellulose is 4.01%, Zn is 31.03 mg/kg (Moyo *et al.*, 2011), saponin

is 80 g/kg (Ferriera *et al.*, 2008), phenol is 8 μ g/mL, flavonoids is 27 μ g/mL (Rajanandh & Kavitha, 2010), and alkaloid is 0.07% (Madukwe *et al.*, 2013), ferulic acid is 46.8 mg/g, and chlorogenic acid is 18.0 mg/g (Fitri *et al.*, 2015). In addition, *Moringa* leaves contain tannin 21g/kg DM and pytat 21g/kg DM that can be ignored as anti-nutrients for ruminants, and do not contain trypsin and amylase inhibitors, lectins, cyanogenic glycosides, and glucosinolates (Ferreira *et al.*, 2008).

Various studies have been carried out by utilizing *Moringa* leaves as feed material, but for the purpose of increasing libido and semen quality is still limited. The results of research of Abu *et al.* (2013) show that *Moringa* leaves meal (MOLM) had no adverse effect on the testicular morphometry and epididymal sperm quality of rabbit bucks at inclusion level of up to 15%. It is suggested that *M. oleifera* leaves can be used in rabbit diets. Raji & Njidda (2014) showed that supplementation with *Moringa* leaves at 50% level of inclusion could enhance the gonadal and extragonadal sperm reserves in Red Sokoto goats. Therefore, this study will use *Moringa* leaves as feed dietary supplement to increase libidos, serum testosterone concentrations, and sperm motilities in Bali bulls.

MATERIALS AND METHODS

Animals, Experimental Design, and Diet

Animals. This study used 4 Bali bulls with 5 years old age and average body weight of 262 \pm 40.40 kg. The experimental bulls were intensively kept under individual animal house.

Experimental design. This study was divided into 2 periods and each period was conducted for 8 weeks. The first 2 weeks of each period were the adaptation phase. At the first period, the experimental bulls were fed with concentrates (1% of body weight) and rice straw *ad libitum* (control). Furthermore, at the second period, they were fed diet similar to that used in the first period with an additional of *Moringa* leaves 15% of the weight of the concentrate (treatments). This study used two-sample t-test to compare each parameter in both periods.

Measurements of libido and semen quality were carried out simultaneously twice a week. Libido was measured by calculating the time required for the bulls from approaching the teaser until they ejaculated. Before semen collection, the false mounting was conducted twice.

Semen was collected by using an artificial vagina. The collected semen was handled according to the procedures of Susilawati (2011). The volume, color, pH, and, viscosity, as well as a mass movement, were recorded. Evaluation of semen quality in the form of the sperm concentration, motility, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN), amplitude of lateral head movement (ALH), and beat cross frequency (BCF) were done by using Computer Assisted Semen Analyzer (CASA) with Sperm Vision™ Version 3.7.5 software.

Blood samples were taken from the jugular vein using vacutainer tubes at the last day for both periods of

study. The samples were collected 3 times a day (06:00, 14:00, and 22:00 h). The plasma samples were stored at -20°C until the hormonal assay. Testosterone was analyzed by using ELISA technique in accordance with the procedures of DRG International, Inc., USA (2009).

Diet and feeding. During the experimental period, the experimental bulls were fed with rice straw *ad libitum* and supplemented with concentrate 1% of their body weight. The experimental bulls were given rations according to the standard nutritional needs for bulls by Nutrient Requirements of Beef Cattle (2000).

Table 1. The composition of feed concentrate

Feed ingredients	%
Rice bran	25
Cocoa pod meal	12
Shrimp heads meal	15
Soybean meal waste/ tofu waste	40
Salt	5
Mineral mix for beef	3
Total	100

Table 2. Nutrient composition of feed concentrate, *Moringa* leaves, and rice straw (as % DM)

Nutrient (%)	Feed concentrate	<i>Moringa</i> leaves	Rice straw
Dry matter	63.62	88.16	89.86
Crude protein	13.47	25.70	4.12
Ether extract	5.64	10.20	1.35
Crude fiber	21.69	9.48	33.25
Nitrogen free extract	34.76	41.56	44.89
Ash	24.76	13.06	16.39
Ca	2.53	3.34	0.46
P	0.67	0.39	0.10

Note: Analyzed by Laboratory of Nutrition and Animal Feedstuff, Faculty of Animal Sciences, Hasanuddin University.

Table 3. Composition of experimental ration (%)

	Control	Treatment
Ingredient (%)		
Rice straw	88	85
Concentrate	12	12
<i>Moringa</i> leaves	0	3
Nutrient composition (%)		
Dry matter	85.64	85.40
Crude protein	5.24	5.87
Ether extract	1.87	2.13
Crude fiber	31.86	31.16
Nitrogen free extract	43.67	43.52
Ash	17.4	17.36
Ca	0.71	0.80
P	0.17	0.18

Composition of concentrate and chemical composition of concentrate, *Moringa* leaves, and rice straw are presented in Table 1 and Table 2. Composition of experimental ration is presented in Table 3.

Statistical Analysis

Data (sperm concentration and motility) were reported as mean and standard error of mean analyzed using the paired student's t-test with SPSS® Version 22 software.

RESULTS

Dry Matter and Nutrients Consumptions

The dry matter and nutrients consumptions of experimental Bali bulls are presented in Table 4. The dry matter and TDN consumptions of experimental Bali bulls supplemented with *Moringa* leaves were not statistically different compared to the control Bali bulls without *Moringa* leaves supplementation. However, the crude protein, Ca, and P minerals consumptions were significantly higher ($P < 0.05$) in the experimental Bali bulls supplemented with *Moringa* leaves compared to control.

Testosterone Levels and Libido

Diurnal changes of plasma testosterone concentrations and libido of the experimental Bali bulls are presented in Figures 1 and 2. Plasma testosterone concentrations in the experimental Bali bulls supplemented with *Moringa* leaves were higher in the morning, at noon, and in the evening compared to the control group. Libido of the experimental bulls supplemented with *Moringa* leaves was significantly higher ($P < 0.05$) compared the control group.

Semen Quality and Characteristics of Sperm Motility of Bali Bulls

Semen quality (sperm concentration and motility) and characteristics of sperm motilities in experimental Bali bulls are presented in Table 5. The concentrations of sperm of experimental Bali bulls supplemented with *Moringa* leaves were not statistically different compared

Table 4. Dry matter and nutrients consumptions of Bali bulls without and with supplementation of *Moringa* leaves (kg/day)

Intake	Control	Treatment	Requirement ¹
Dry matter	13.86±0.271	13.29±0.271	6.30
Crude protein	0.72±0.011 ^a	0.78±0.012 ^b	0.59
TDN	6.03±0.12	5.79±0.12	3.67
Ca	0.098±0.002 ^a	0.107±0.002 ^b	0.015
P	0.023±0.0003 ^a	0.024±0.0003 ^b	0.009

Note: Means in the same row with different superscripts differ significantly ($P < 0.05$). ¹Adapted from NRC (2000), Nutrient Requirements of Beef Cattle, 7th revised edition.

to the control Bali bulls without *Moringa* leaves supplementation. The total and progressive motilities of sperm were significantly higher ($P < 0.05$) in the experimental Bali bulls supplemented with *Moringa* leaves compared to the control Bali bull without *Moringa* leaves supplementation.

The velocities of sperm in a minute on the trajectory curve (VCL), on a straight path (VSL), and the average trajectory of the plot (VAP), were significantly faster ($P < 0.05$) in the experimental Bali bulls supplemented with *Moringa* leaves compared to the control groups. The percentage of LIN was significantly lower ($P < 0.05$) in the experimental Bali bulls supplemented with *Moringa* leaves compared to the control groups. Distance lateral movement of the sperm head at each average plot (ALH) was significantly higher ($P < 0.05$) in the experimental Bali bulls supplemented with *Moringa* leaves compared to the control Bali bulls without *Moringa* leaves supplementation. However, the frequency of sperm movement (BCF) or the average flow linear curve average sperm pass through the plot was similar in experimental Bali bulls with or without *Moringa* leaves supplementation.

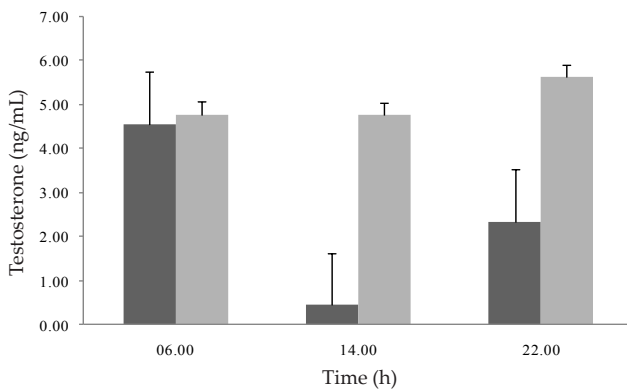


Figure 1. Diurnal changes of plasma testosterone concentrations in Bali bulls without (control; dark bar) and with (treatment; pale bar) supplementation of *Moringa* leaves.

DISCUSSION

The results of this study indicated that supplementation of Bali bulls with *Moringa* leaves increased plasma testosterone concentrations, libido, and sperm motility. During the treatment period (period 2), the plasma testosterone concentrations were higher all the day long, the intervals between approaching the teaser until ejaculation were shorter, and the total and progressive motilities were higher compared to those during the untreated period.

The higher plasma concentrations of testosterone during the *Moringa* leaves supplementation was in accordance with the study in rats by Prabsattroo *et al.* (2015) and Dafaalla *et al.* (2016). They reported that *Moringa* leaves supplementation significantly increased serum testosterone in the rat. The treatment of *Moringa* leaves could also increase Leydig cells (Prabsattroo *et al.*, 2015) and the levels of FSH and LH (Dafaalla *et al.*, 2016). The testosterone synthesis performed by Leydig cells in the testes depends on the adequacy of Zn in the diet (Roy *et al.*, 2013). Further, Zn minerals stimulate Leydig cells to produce testosterone for the

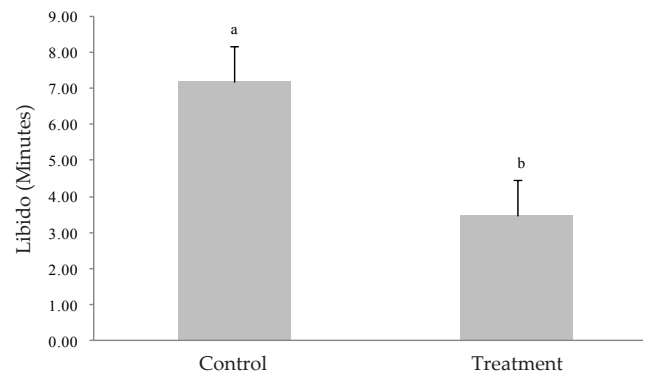


Figure 2. Libido of the Bali bulls without (control) and with (treatment) supplementation of *Moringa* leaves.

Table 5. Sperm concentration, motility, and characteristic sperm motility of Bali bulls without and with supplementation of *Moringa* leaves

Variables	Without supplementation (Control)	With supplementation (Treatment)	References
Concentration (million/mL)	791.00 ± 96.80	817.00 ± 58.48	300–2,000 ¹
Total motility (%)	63.99 ± 3.37 ^a	84.96 ± 3.09 ^b	70–90 ²
Progressive motility (%)	52.77 ± 1.76 ^a	67.03 ± 3.74 ^b	>50 ²
Characteristic motility:			
VCL (μm/s)	71.78 ± 2.07 ^a	91.24 ± 4.32 ^b	121.04 ± 18.10 ³
VSL (μm/s)	31.18 ± 0.48 ^a	35.14 ± 0.84 ^b	60.39 ± 14.65 ³
VAP (μm/s)	43.10 ± 1.05 ^a	51.66 ± 1.70 ^b	73.19 ± 13.42 ³
LIN (%)	0.44 ± 0.01 ^a	0.39 ± 0.01 ^b	0.49 ± 0.06 ³
ALH (μm)	5.47 ± 0.12 ^a	6.23 ± 0.20 ^b	4.19 ± 0.52 ³
BCF (Hz)	19.37 ± 0.25	19.70 ± 0.28	30.24 ± 5.54 ³

Note: Means in the same row with different superscripts differ significantly ($P < 0.05$). References: ¹Setchell (2014), ²Susilawati (2011), and ³Sarastina *et al.* (2012).

normal function of the hypothalamus-pituitary-testes axis. The lack of Zn causes the damage of LH receptor mechanisms in controlling, storing, and releasing of testosterone. Kumar *et al.* (2006) showed that Zn supplementation significantly increased serum testosterone in crossbreed cattle (*Bos indicus* × *Bos taurus*) bulls. A similar results were reported by Roy *et al.* (2013) in cattle, buffalo, and rabbit. Therefore, the high Zn level in *Moringa* leaf used in this study was considered to be one of the possibilities that affected the higher level of testosterone during the supplementation with the *Moringa* leaves. This condition is also supported by the results reported by Kumar *et al.* (2006) that Zn is an important component of proteins involved in the synthesis and secretion of testosterone. The results of this study indicated that supplementation of *Moringa* leaves significantly increased the consumption of crude protein, so that high protein content in *Moringa* leaves can also be suspected to trigger the synthesis process and secretion of testosterone during the treatment period.

Libido (time needed for ejaculation) of Bali bulls during the supplementation of *Moringa* leaf was shorter compared to that in the control i.e., 3.49 vs 7.20 min. Average libido of Bali bulls is 4.5 min (Susilawati, 2011). Since libido is affected by testosterone levels (Prabsattroo *et al.*, 2012 and Roy *et al.*, 2013), the increased libido of experimental Bali bulls supplemented with *Moringa* leaves in this study was due to the elevated levels of testosterone. Therefore, the supplementation of *Moringa* leaves in the ration of bull could increase the level of testosterone and then increased the libido.

The results of this study indicated that supplementation of *Moringa* leaf could maintain sperm concentration of Bali bulls under a normal level. The sperm concentration found in this study was similar to that reported in the same breed of bulls by Susilawati (2011) and Setchell (2014). The failure of *Moringa oleifera* to increase the sperm concentration reported in this study was in contrary to that reported in mice by Priyadarshani & Varma (2014) and in rats by Prabsattroo *et al.* (2015). They found that in hyperglycaemic mice and stressed rats, the supplementation of *Moringa* leaves significantly increased sperm concentration. This discrepancy might be due to the level of intake of *Moringa* leaves compared to body weight.

The supplementation of *Moringa* leaves in this study increased the total and progressive motilities of sperm in the Bali bulls. The total and progressive motilities of the sperm in this study was similar to those reported for the same breed of bulls at Singosari Artificial Insemination Center (BBIB) i.e., $83.46 \pm 4.27\%$ and $70.68 \pm 8.92\%$ (Sarastina *et al.*, 2012). Study of Eghbali *et al.* (2010) showed that the total Ca content of seminal plasma of the buffalo is highly correlated with the total and progressive motilities of the sperm. Princewill *et al.* (2015) suggested that P is necessary for sperm motility. The phosphorus concentration detected in the seminal plasma of active bulls was positively correlated with both quantity and quality parameters of bovine semen. The results of this study indicated that supplementation of *Moringa* leaves significantly increased Ca and P consumption, so that high Ca and P content in *Moringa*

leaf can be suspected as the cause of increased sperm motility during the period of *Moringa* leaves supplementation. In addition, study of Begum *et al.* (2009) showed that some nutrients that contribute to sperm motility were arginine, carnitine, Zn, vitamin B12, vitamin C, vitamin E, glutathione, selenium, and Coenzyme Q-10. The nutrients found in *Moringa* leaf are arginine, Zn, Se, vitamin C, and E. Since *Moringa* leaf contains amino acid arginine (1.78%), Zn (31.03 mg/kg), Se (363 mg/kg) (Moyo *et al.*, 2011), vitamin C (220 mg/100g) (Dhakar *et al.*, 2011), and vitamin E (77 mg/100 g) (Moyo *et al.*, 2011), the higher sperm motility in bulls fed with *Moringa* leaves might be due to these nutritional contents. Further studies are needed to clarify the main nutrients that significantly affect the sperm motility.

The supplementation of *Moringa* leaves increased velocity (VCL, VSL, and VAP) and amplitude (ALH), but decreased linearity (LIN) of sperm in Bali bulls. Nevertheless, the values obtained are still lower than those found in Bali bulls in BBIB Singosari, probably because the bulls in BBIB Singosari are superior and selected bulls and the bulls are fed with quality feed. According to Susilawati (2011), there are 3 patterns of sperm motility, namely hyperactivate, non-hyperactivate, and transition groups. Fertility rates in the hyperactivate group had a higher success compared to the non-hyperactivated group. The requirements for hyperactivate are VCL values $\geq 100 \mu\text{m/s}$, LIN $< 60\%$, and ALH $\geq 5 \mu\text{m}$. The observed sperm has not been included in the hyperactive category, but is approaching hyperactivate, because the VCL value is only $91.24 \mu\text{m/s}$.

The characteristics of sperm motility were positively correlated with fertility (Perumal *et al.*, 2014), so it can be used to predict the fertilization capacity of the sperm. VAP, VSL, and LIN values are progressive motility indicators and VCL, ALH, and BCF values are indicators of sperm rigor. VAP and VCL values are a good predictor of the ability of sperm to fertilize *in vitro* (Susilawati, 2011). The values of these parameters at the treatment period were higher than during pretreatment or control period, thus having the possibility of producing more fertile sperm.

CONCLUSION

The supplementation of 15% *Moringa* leaves in the concentrate (1% of body weight) markedly increased plasma testosterone concentrations, libido, and sperm motility of Bali bulls.

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